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by

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Subject: Harmful algal blooms and Pfiesteria piscicida

My name is Lynn Donelson Wright and I am Dean and Director of the Virginia Institute of Marine Science of the College of William and Mary. The Virginia Institute of Marine Science, School of Marine Science (VIMS/SMS) was established in 1940 as the Virginia Fisheries laboratory. The Virginia Institute of Marine Science has as its mission research, education, and advisory service to support the needs of the Commonwealth of Virginia and the Nation. The emphasis is on interdisciplinary coastal and estuarine marine science. In a society that is

increasing its pressure on the environmental and natural resources, the coastal ocean and estuarine environment is a region of vital concern. The Institute's central purpose is to provide sound, objective scientific knowledge and advice in support of management and policy as applied to the marine resources of the Commonwealth of Virginia specifically and the coastal ocean generally. It is internationally and nationally recognized as an unbiased source of objective scientific advice, and it is respected by government, industry and the general public for the quality of that expertise.

Among its many research activities, the Institute is presently serving as a member of the Virginia *Pfiesteria* Task Force and is actively engaged in research aimed at improving understanding of this enigmatic organism as quickly as possible within the constraints imposed by accepted scientific rigor. VIMS has a multi-faceted involvement in *Pfiesteria*-related research that includes both field and laboratory studies. Rigorous laboratory studies are critical because much information inferred from field observations has not yet been verified or firmly established by careful laboratory studies. There is a critical need for research on a wide variety of topics related to *Pfiesteria* and other *Pfiesteria*-like organisms.

During the summer of 1997, the presence of lesions on finfish and a modest fish kill, involving mostly juvenile menhaden, signaled the potential presence of toxic *Pfiesteria piscicida* and related dinoflagellates in Chesapeake Bay. Although these episodes were the region's most notable experience with harmful algal blooms, such phenomena are increasingly common in estuarine and coastal waters, both nationally and internationally, and are recognized as a growing threat to coastal living resources, economies and public health.

The cause(s) of harmful algal blooms and their increasing prevalence are not well understood and are likely varied, but there is growing concern that they may be aggravated by human activities. For example, increased nutrient inputs, primarily forms of nitrogen and phosphorus, from land to

coastal waters are suspected as an important factor. Because of the potential risk that harmful algal blooms in general and *Pfiesteria piscicida* in particular may pose to human health, natural resources and environmental quality, there is an increased urgency within various federal and state agencies to support research programs in relevant areas.

Much is known about *Pfiesteria piscicida*, but there is still much to learn before we can develop effective management and mitigation strategies or predict outbreaks of this organism. In addition, there are other species in the *Pfiesteria* complex and the basic biology and toxicity of these other species is not well studied. The species present in any given estuary or coastal system and their spatial and seasonal distributions are not well documented, but more than one species has been found in most areas. Clearly, we need much more research on species in the *Pfiesteria* complex, on their impact on human health and living marine resources and on the ecological factors, including nutrient inputs, that control their abundance and expression of toxicity.

What is Virginia currently doing in regard to *Pfiesteria*?

- 1. VIMS conducts monthly trawl and seine surveys in Virginia waters. These surveys document the temporal and spatial occurrence of fish with lesions in Virginia and are an effective early-warning system for the potential presence of toxic stages of *Pfiesteria*-like organisms.
- **2.** VIMS has established a Web site to provide public access to a fact sheet on *Pfiesteria*, the Task Force, periodic updates released by the Virginia Health Department and links to other web sites with information on *Pfiesteria*.
- **3.** VIMS scientists have perfected protocols for culturing non-toxic stages of *Pfiesteria*-like organisms and for identifying them using scanning electron microscopy. VIMS now serves as an identification facility for Virginia.
- **4.** With funding from the Commonwealth of Virginia, VIMS scientists are initiating a comprehensive ecological study in the Great Wicomico River to investigate the relationships among *Pfiesteria* abundance, fish with lesions, nutrients and other water quality parameters in an attempt to understand the factors that regulate *Pfiesteria* abundance and the expression of toxicity.
- **5.** The Virginia Department of Health is initiating a cohort study to determine human health effects of outbreaks of Pfiesteria-like organisms. This study is funded by CDC and is supported by VIMS (fish lesion monitoring and *Pfiesteria* identification), Old Dominion University (initial screening of water samples for *Pfiesteria*-like organisms) and the Department of Environmental Quality (water quality parameters).
- **6.** The Virginia Department of Environmental Quality has developed protocols for rapid response to a *Pfiesteria*-related fish kill. In addition, with funding from EPA, DEQ has expanded their water quality monitoring program to include screening for *Pfiesteria*-like organisms.

What is *Pfiesteria*?

Pfiesteria and related organisms are dinoflagellates, microscopic, free-floating, single-celled organisms with two flagella for locomotion. *Pfiesteria* is not a virus or bacterium and it is not an infectious agent; fish or other organisms cannot become infected with *Pfiesteria*. Most dinoflagellates are plants (called algae or phytoplankton) that gain energy from photosynthesis. However, many species of dinoflagellates, including *Pfiesteria*, do not normally photosynthesize, but behave more like animals and consume algae or bits of organic matter. *Pfiesteria* piscicida is known to have a highly complex life cycle with 24 identified forms. The three typical forms are the flagellated stage, a benthic amoeboid form and an encysted stage.

Identification of *Pfiesteria*-like organisms is difficult and can be accomplished with certainty only with the use of a scanning electron microscope. Light microscopy is unreliable for identification. It is now recognized that there are many separate species in the *Pfiesteria*-like complex. The exact number is not known at present and the toxicity is unknown for most of these other species.

What causes *Pfiesteria* and related forms to become toxic?

Pfiesteria normally exists in non-toxic forms, feeding on algae and bacteria in the water and in sediments. The conditions that trigger the transformation from a non-toxic to a toxic stage are not well understood, but it seems to occur only in the presence of fish. It is thought that secretions or excretions from fish somehow trigger the expression of toxicity. Toxic outbreaks of *Pfiesteria* seem to depend on hydrographic conditions that allow the organisms to detect fish secretions and also prevent rapid flushing of the toxin. These conditions occur in shallow, poorly flushed systems.

Does *Pfiesteria* occur in Chesapeake Bay?

Pfiesteria piscicida has been confirmed from tributaries in Maryland and probably exists throughout Chesapeake Bay in areas of appropriate salinity. Two other organisms now recognized as close relatives of *Pfiesteria* have also been reported from Chesapeake Bay.

What causes lesions (open sores) on fish?

There are many possible causes for fish lesions including physical injury in nets or traps, bites by other fish or birds, toxic chemicals, and diseases such as viruses and bacteria. On the basis of laboratory experiments, we now have to add toxins released by *Pfiesteria* to the list of possible causes. The present state of scientific knowledge is usually insufficient to allow determination of the original cause of a lesion unless an obvious parasite is present.

Open sores that invade the musculature are the most difficult to assess. The skin and mucus of a fish are effective barriers against infection by bacteria, which are always present in Chesapeake Bay waters. However, that barrier can be broken by a variety of causes including injury, general stress or toxic chemicals (including *Pfiesteria* toxins). When the skin/mucus barrier is broken, the area is usually rapidly colonized by bacteria that further erode the tissue and produce an open lesion or sore that may

penetrate deep into the musculature. In such cases, our present state of knowledge is usually not sufficient to determine what caused the original break in the skin/mucus barrier that lead to the lesion.

Were there unusually high numbers of fish lesions in Chesapeake Bay during 1997?

No, not in most areas of Chesapeake Bay. Some fish lesions occur every summer in Chesapeake Bay and based on information from VMRC, DEQ and VIMS and also from agencies in Maryland, the incidence of lesions on Chesapeake Bay fish during 1997 was not unusually high and there is no indication that fish populations are facing serious problems.

The Pocomoke River, located on the Eastern Shore near the Virginia-Maryland border was an exception. Commercial fishermen reported what they consider to be unusually high numbers of fish lesions in the Pocomoke River and there were low- to moderate-level fish kills in the river during August of 1997. These lesions and kills were linked to the toxic dinoflagellate *Pfiesteria piscicida* and related forms.

Are fish kills known to occur in Chesapeake Bay?

Small- to moderate-scale fish kills, usually of small menhaden, occur occasionally in tidal creeks during the summer months. These kills are usually caused by low oxygen content of the water, but other possible causes, including *Pfiesteria*, are routinely investigated.

Can Chesapeake Bay expect large-scale fish kills similar to North Carolina?

When fish with lesions were first observed in the Pocomoke River there was doubt about the possible role of *Pfiesteria* as a cause because of the lack of large numbers of dead fish on the surface. In North Carolina, where *Pfiesteria* has been reported to be the cause of fish kills, there are reports of large numbers of dead fish, often hundreds of thousand to millions, during fish kills. Fish kills in the Pocomoke River, attributable to *Pfiesteria*, report thousands to perhaps tens of thousands of dead fish, much lower numbers than observed in North Carolina.

One possible explanation for the fewer numbers of dead fish in the Chesapeake Bay region as a result of *Pfiesteria* may be differences in hydrography between these two regions. The Pamlico Sound and Nuese River estuary in North Carolina are very shallow, poorly flushed estuaries with weak tidal currents. It is possible that the greater the dispersion of these chemical cues and toxins by water currents and circulation, the fewer fish will be detected and killed. Also, in deeper water fish may be less concentrated.

Is it safe to eat Virginia seafood?

YES, Chesapeake Bay seafood is safe. Consumers should use common sense and avoid dead fish or fish with sores, but otherwise there is no reason to avoid eating Virginia seafood. There have been no reports of adverse effects on human health from

eating shellfish (crabs, oysters, etc.) harvested in the vicinity of fish kills, but little information is available on this subject.

Is *Pfiesteria* related to red tides?

There are two reasons that the public may connect *Pfiesteria* and "red tides". *Pfiesteria* is a dinoflagellate and red tides are typically, but not always, caused by dinoflagellates. *Pfiesteria* is known to be toxic to fish and red tides are often, but not always, toxic to marine life. Despite these similarities, there are important distinctions to be made between *Pfiesteria* and red tides, especially for the Chesapeake Bay region. *Pfiesteria* is reported to kill fish when it occurs at low concentrations in the water, typically a few hundred cells per milliliter (.00026 gallons) of water. This is not a sufficient concentration of cells to discolor the water and *Pfiesteria* has never been reported to cause discolored water.

Red tides (also called red water or mahogany water) are typically caused by the dense accumulation, typically thousands of cells per milliliter of water, of dinoflagellates near the surface. Red tides are common occurrences in the Chesapeake Bay and its tributaries. They can occur at any time of year but usually are most common during July and August. Unlike other coastal regions of the United States where red tides result in fish death and bans on eating shellfish, red tides in the Chesapeake Bay to date have not been toxic to marine life. This lack of toxicity is because the species of dinoflagellates causing red tides in Chesapeake Bay are not toxic species. Red tides are typically categorized as a type of Harmful Algal Bloom (HAB), whether they are harmful to aquatic life or not. There is increasing interest in HABs worldwide because of the perception that they are becoming much more numerous, are often toxic to marine life, and are likely caused by man's influence on coastal areas. Because of their lack of toxicity to date, there has been less urgency to study red tides in the Chesapeake Bay and it is not clear what causes them and whether they are becoming more numerous.

Is there a relationship between *Pfiesteria* and environmental degradation?

Popular press reports of *Pfiesteria* and its possible effect on fish often suggest that nutrient enrichment of estuaries and coastal waters from a variety of land-derived sources is a principal cause of *Pfiesteria* proliferation and activity. Some scientific literature suggests a similar relationship. Manure from hog and chicken production facilities is often identified as a source of nutrients. The association between *Pfiesteria* and nutrient enrichment is also fostered by the tendency to associate *Pfiesteria* with algal blooms, which are well documented to result, in part, from nutrient enrichment of natural waters. However, as discussed above, *Pfiesteria* is not an algae and does not make its own food by photosynthesis and does not require dissolved nitrogen and phosphorous (two typical nutrients) in the water for its nutrition. *Pfiesteria* eats other microscopic plants and animals. Because it is an animal and not a plant it is less likely to respond directly to nutrient enrichment. To the extent that its preferred food is microscopic algae, one might expect *Pfiesteria* to be more abundant where its preferred food is more abundant. Thus, it might be indirectly linked to nutrient enrichment through its food supply. Some evidence suggests that nutrients, especially organic

forms, may stimulate the growth of *Pfiesteria* directly. However, more research is needed to show this conclusively and to determine which nutrients and which form of nutrients are involved.

In general, the Chesapeake Bay and its tributaries are not as enriched with nutrients as the Pamlico Sound and its tributaries in North Carolina, yet *Pfiesteria* has been reported from various locations in the Chesapeake Bay and has been linked to fish kills and human health problems in the Pocomoke River. Until more research results are available it is not possible to say with confidence why *Pfiesteria* occurs where it does and why it becomes toxic when and where it does.

Does *Pfiesteria* affect humans?

A variety of symptoms have been reported by commercial watermen and other citizens in North Carolina, Maryland and Virginia and by researchers who cultured *Pfiesteria* in the laboratory. Symptoms, including sores, fatigue and short-term memory loss, have only been associated with laboratory exposure, or with large-scale fish kills in North Carolina and with fish kills in the Pocomoke River in Maryland and Virginia. Portions of the Pocomoke River were closed periodically during August because of possible human health concerns. Establishing a definite link between generalized symptoms and *Pfiesteria* is difficult, but health officials are studying the situation carefully. Unless an area has been closed by the Health Department, there is no reason to fear swimming or boating in Chesapeake Bay.

How would additional funding be utilized to study toxic dinoflagellates?

The Federal Government has provided much needed funding support for research on harmful algal blooms and *Pfiesteria*. The multi-agency program entitled OEcology and Oceanography of Harmful Algal Blooms (ECOHAB) has provided competitive research funding for harmful algal blooms and the budget for this program was increased during 1998 to include specific funding for *Pfiesteria*. In addition the Centers for Disease Control has received funding that will allow impacted coastal states to study human health effects of *Pfiesteria* and related organisms. Other Federal funding has been provided by EPA and NOAA to support a variety of training workshops related to *Pfiesteria* and to expand state monitoring for outbreaks of *Pfiesteria*-like organisms.

This funding has facilitated increased research and monitoring in relation to harmful algal blooms and *Pfiesteria*. However, much more research needs to be accomplished before effective management and mitigation strategies can be developed and implemented and before we are able to predict the onset of blooms or outbreaks. In addition, state monitoring programs must be augmented to protect human health. Therefore, I fully support Senate Bill 1480. It provides substantial increases in competitive research funding through the ECOHAB and Coastal Zone Management programs, it provides funding for outreach, education and advisory services through the National Sea Grant College Program and it provides assistance for annual monitoring by impacted coastal states

There are four primary areas of research that are important for a full understanding of the biology of toxic dinoflagellates and their effects on living marine resources.

- 1. Rapid identification methods. A number of heterotrophic dinoflagellates are recognized in what is now referred to as the *Pfiesteria* complex or *Pfiesteria*-like species. At the present time, these species can be identified only with the aid of a scanning electron microscope. It is critical that rapid, sensitive, state of the art molecular or immunological diagnostic techniques be developed for these organisms. This will eliminate the tedious and time-consuming electron microscopy for routine diagnosis and will greatly improve the ability to rapidly identify *Pfiesteria*-like organisms in water samples. The studies listed below will have to be completed for each species identified.
- **2. General biology and nutritional ecology of** *Pfiesteria***-like organisms.** The general biology of *Pfiesteria*-like organisms is poorly understood. A number of different life cycle stages have been reported for *Pfiesteria piscicida*, but the factors that trigger transformation from one stage to another are not understood and it is not known if other similar species have similar life cycle stages. Basic environmental tolerance to temperature, salinity and pH are not known for most *Pfiesteria*-like organisms. Of particular importance is the nutritional ecology of *Pfiesteria*-like organisms. On the basis of field observations in North Carolina, a link has been suggested between degraded water quality and abundance of *Pfiesteria*-like organisms. However, this relationship has not been well established in laboratory experiements. Careful laboratory studies are critically needed to establish this relationship with certainty, including studies to examine the relationship between abundance of *Pfiesteria*-like organisms and organic and inorganic nutrient enrichment.
- **3. Toxin characterization and toxicity studies.** Each species in the *Pfiesteria* complex probably has a different toxin. It is critical that these be purified and characterized so that their mode of action can be determined and so that rapid detection tests can be developed to allow measurement of toxin levels in the water and in tissues of aquatic organisms. Purification and characterization of the toxin will also allow studies on the effect of toxins on marine organisms. For example, although it is known that *Pfiesteria* toxins can cause degradation of the skin of fishes, the relationship between Pfiesteria-like toxins and lesions on fish is poorly understood and there is not at present a definitive *Pfiesteria* lesion that can be identified. Careful laboratory studies are needed to determine the relationship between *Pfiesteria*-like organisms and fish lesions with certainty. This relationship may vary among the various species in the *Pfiesteria* complex. The conditions under which *Pfiesteria*-like organisms transform from a non-toxic to a toxic state are poorly understood. Toxin production seems to require the presence of fish, but other factors, including hydrodynamics, are undoubtedly important. A thorough study of these factors is necessary to identify areas where *Pfiesteria*-like organisms are likely to occur. Similarly, human health effects from *Pfiesteria*-like organisms are poorly understood, but could be investigated using mammalian models if toxins were purified and characterized.
- **4. Epidemiology.** Understanding the spatial and temporal distribution of lesions on juvenile menhaden and other fishes in relation to abundance of *Pfiesteria*-like organisms is critical to a full understanding of the problem. Analyses of spatial and temporal patterns of lesion abundance in relation to *Pfiesteria* abundance, salinity, nutrient levels and other environmental parameters will allow formulation of hypotheses on controlling factors that can then be examined in laboratory or

studies. In addition, such studies may allow development of an early warning system for *Pfiesteria* outbreaks.

Because of extensive studies along the entire southeastern coast of the U. S. during the menhaden lesion problem of the mid 1980s, some historical data are available that can be used to examine the relationship between fish lesion/*Pfiesteria* abundance and long-term climatic effects.